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# Protection Against Nuclease Cleavage of pBR322 DNA by the cAMP Receptor Protein of *Escherichia coli*

The effect on nuclease digestion of DNA exerted by the cAMP receptor protein (CRP) of *Escherichia coli* has been investigated. It is shown that non-specific binding of CRP to double-stranded supercoiled pBR322 DNA protects the DNA against digestion by the restriction endonucleases *Eco*RI, *Bam*HI and *Bgl*I, and the nuclease DNAase I.

The cAMP† receptor protein mediates the regulation of a number of catabolite-sensitive operons in *Escherichia coli*, cAMP acting as an allosteric effector (Zubay *et al.*, 1971; Epstein *et al.*, 1975; de Crombrugghe & Pastan, 1978). The cAMP·CRP complex interacts with specific DNA sites near promoters, stimulating the initiation of messenger RNA synthesis (de Crombrugghe *et al.*, 1971; Majors, 1975; Taniguchi *et al.*, 1979; Odgen *et al.*, 1980). CRP is a dimer of molecular weight 45,000, composed of two apparently identical subunits (Anderson *et al.*, 1971; Riggs *et al.*, 1971). The X-ray crystal structure of the cAMP·CRP complex has been determined at 2·9 Å resolution, and shows that each subunit consists of two structural domains: the larger amino-terminal domain contains the cAMP binding site and the smaller carboxy-terminal domain is presumed to contain the DNA binding site (McKay & Steitz, 1981). Electron microscopic studies have shown that co-operative non-specific binding of CRP to double-stranded linear DNA (Takahashi *et al.*, 1979; Saxe & Revzin, 1979) results in DNA condensation and the formation of regularly striated fibres (Chang *et al.*, 1981). We report here that, probably due to the formation of this ordered fibre structure, CRP protects double-stranded supercoiled DNA from cleavage by several restriction endonucleases and DNAase I.

Figure 1 shows the results of electrophoresis, after digestion by *Bgl*I, *Bam*HI and *Eco*RI, of pBR322 DNA in the presence and absence of CRP. Although protection against cleavage was not complete (tracks IIb, IIIb and IVb contain a small amount of linearized plasmid DNA), there is a remarkable difference between digestion without (a tracks) and with (b tracks) CRP present.

In the absence of CRP we observe that digestion by *Eco*RI (track IIa) leads to complete linearization of unprotected DNA; digestion by *Bam*HI (track IIIa) leads to almost complete linearization of unprotected DNA, only a small proportion being left as supercoiled dimer; and digestion by *Bgl*I (track IVa), however, leads to only a small amount of cleavage product, due to the low

† Abbreviations used: cAMP, adenosine 3′,5′-cyclic phosphate; CRP, cAMP receptor protein of *E. coli*; DNAase I, deoxyribonucleate 5′-oligonucleotido-hydrolase (EC 3.1.4.5) from bovine pancreas.



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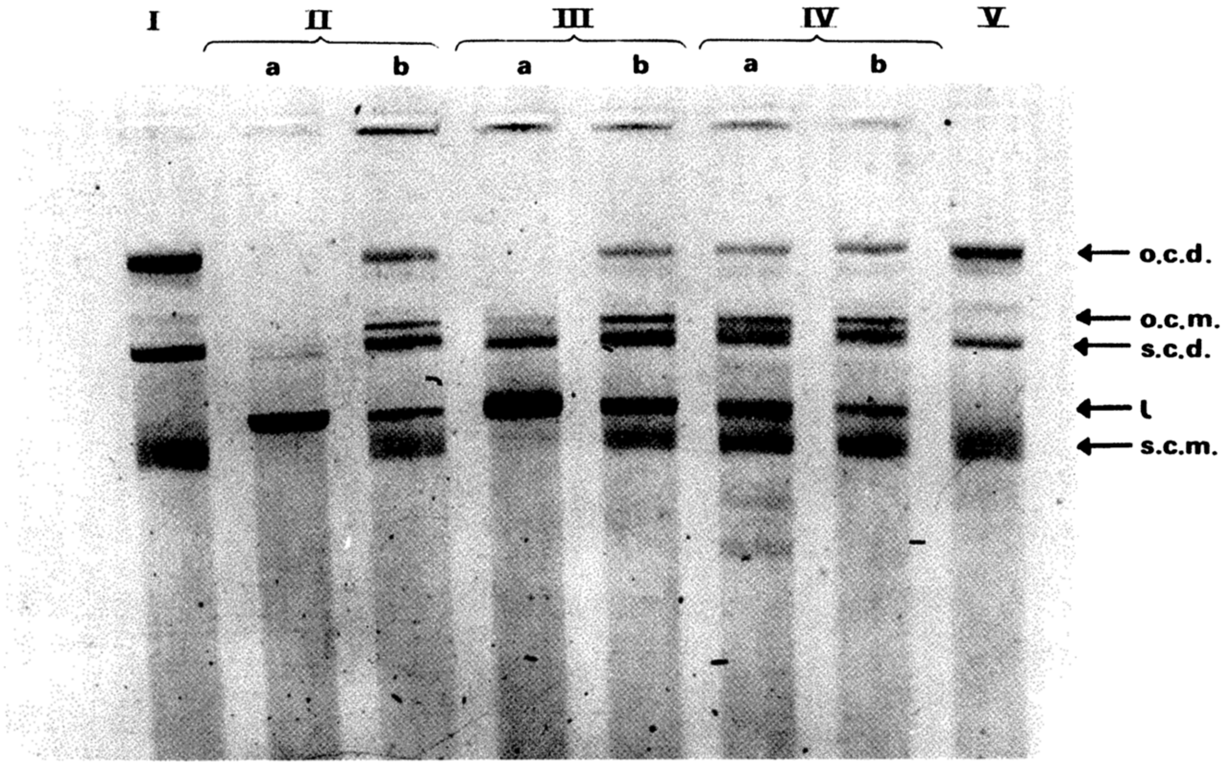


FIG. 1.

activity of the *BglI* enzyme employed; two bands migrate corresponding to linear double-stranded DNA with lengths of approximately 2700 and 1400 base-pairs (the small fragment of 230 base-pairs is not visible on this 1% agarose gel).

In contrast to the large amount of cleavage product in the absence of CRP (a tracks), there is very little digestion in the presence of CRP under conditions where the DNA lattice is completely saturated with CRP (b tracks). All circular DNA forms are still visible and only a small amount of linearized DNA is detectable. Thus it is clear that CRP, upon binding to pBR322 DNA, prevents restriction endonucleases from cleaving the DNA. That this protection is not due to an irreversible alteration of the normal pBR322 DNA structure can be shown by comparing the gel pattern of pBR322 DNA loaded directly onto the gel (track V) with pBR322 DNA that was complexed with CRP in the same way as the DNA in the b tracks, but not incubated with restriction endonucleases (track I). The pattern is exactly the same in both cases. Furthermore, it is possible after deprotection (i.e. removal of CRP) of the DNA to cut pBR322 DNA with any restriction endonuclease to yield normal cleavage products.

The analogous experiment in which DNAase I was employed as the nuclease also shows that CRP protects DNA from being degraded. Incubation of 0·8 μg of pBR322 DNA for 15 minutes at 37°C with 0·5 μg DNAase I/ml leads to complete degradation of the DNA, while the presence of CRP in sufficient amounts to saturate the DNA completely prevents this (Fig. 2).

It should be noted that the inability of both the restriction endonucleases and DNAase I to digest the DNA in the presence of CRP bound to the DNA is not due to enzyme inhibition by CRP, as protection only occurs when sufficient CRP is

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FIG. 1. Electrophoresis in a 1% agarose gel of 0·4 μg pBR322 DNA, following digestion with restriction endonucleases in the presence and absence of CRP.

Track I, pBR322 DNA with CRP; track II, pBR322 DNA digested by *Eco*RI without (a) and with (b) CRP present; track III, pBR322 DNA digested by *Bam*HI without (a) and with (b) CRP present; track IV, pBR322 DNA digested by *BglI* without (a) and with (b) CRP present; track V, 0·16 μg pBR322 DNA loaded directly onto the gel.

The experiment was carried out as follows: 0·4 μg of pBR322 DNA (prepared by a modification of the procedure of Clewell & Helinski, 1959) was incubated for 10 min at room temperature with 0·28 μmol of CRP (prepared as described by Takahashi *et al.*, 1980) in a total volume of 10 μl of the appropriate buffer for the subsequent restriction endonuclease reaction. Under these conditions the DNA lattice is completely saturated with CRP (Takahashi *et al.*, 1979). Samples (10 μl) of 1 in 100 dilutions of *BglI* (a gift from P. Gilette), *Bam*HI (Boehringer Corp.) or *Eco*RI (Biolabs), respectively, were added and the mixtures were incubated at 37°C for 30 min. The restriction enzymes were inactivated by heating the samples to 65°C for 30 min. Afterwards, 40 μl proteinase K (Sigma) solution (500 μg/ml) were added to destroy the DNA–CRP complex by degrading the protein, and the mixture was kept at 37°C for 30 min followed by 30 min at 65°C. Omitting the proteinase K step leads to loss of all DNA from the aqueous phase, probably because the protein is so tightly bound to the DNA that the protein–DNA complex goes into the phenol phase during the phenol-extraction step. All samples were extracted twice with phenol, and once with chloroform and the DNA was precipitated with ethanol. The dried pellets were taken up in 10 μl $H_2O$. 10 μl loading buffer containing 30% Ficoll and orange G as dye was added and the mixture was electrophoresed on a 1% agarose gel in 40 mM-Tris, 5 mM-sodium acetate, 1 mM-EDTA (pH 7·8) buffer for 2 h at room temperature. The DNA was visualized by staining the gel with ethidium bromide, which was added to the electrophoresis buffer at 0·5 μg/ml, and illuminating from below with ultraviolet light. The gel was photographed with a Polaroid MP-4 camera by using a red filter and a Polaroid 55 film. o.c.d., open circle dimer; o.c.m., open circular monomer; s.c.d., supercoil dimer; l, linear; s.c.m., supercoil monomer.

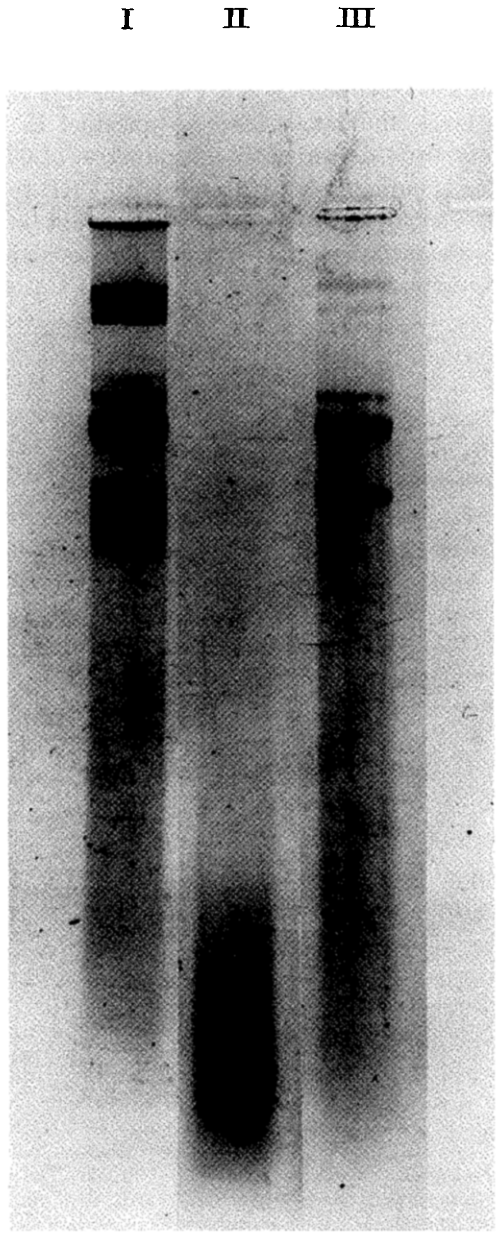


FIG. 2. Electrophoresis in a 1% agarose gel of 0·8 $\mu$g of pBR322 DNA following treatment with DNAase I in the presence of CRP.

Track I, pBR322 DNA loaded directly onto the gel; track II, pBR322 DNA treated with DNAase I in the absence of CRP; track III, pBR322 DNA treated with DNAase I in the presence of CRP.

The experiment was carried out as follows: 0·8 $\mu$g of pBR322 DNA was incubated with 0·56 $\mu$mol of CRP in 10 mM-Tris, 100 mM-KCl, 10 mM-$MgSO_4$, 0·1 mM-EDTA (pH 7·4) for 10 min at room temperature in a total volume of 19 $\mu$l. Under these conditions the DNA lattice is completely saturated with CRP (Takahashi *et al.*, 1979); 1 $\mu$l of DNAase I (Boehringer Corp.) solution (10 $\mu$g/ml) was added and the solution was incubated for 20 min at 37°C: 40 $\mu$l of proteinase K solution (500 $\mu$g/ml) were added and the mixture kept at 37°C for 50 min. The samples were subsequently treated as described in the legend to Fig. 1.

employed to saturate the DNA lattice completely; the use of less CRP leads to cleavage of the DNA by the nucleases, due to incomplete protection.

These experiments show that CRP, probably due to its highly co-operative non-specific DNA-binding ability (Takahashi *et al.*, 1979; Saxe & Revzin, 1979; Chang *et al.*, 1981), is capable of completely enveloping native double-stranded DNA and thus protecting it from nuclease attack. This finding is entirely consistent with the model for non-specific binding of CRP to supercoiled DNA put forward by Salemme (1982), in which CRP envelops a left-handed solenoidal coil of right-handed *B* DNA, with the two antiparallel F helices of CRP bridging the major grooves of adjacent loops of the coil.

The results reported in this paper may provide a basis for the possibility of using CRP to protect foreign DNA during its introduction into cells in which high internal nuclease activity leads to rapid degradation of the foreign DNA.

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